functional expression achieved through the manipulation (Table 1) are in line with the level of expression that has been suggested to be required to reverse the cystic fibrosis defect (Johnson *et al.*, Nature Gen. 2:21-25(1992)).

Patch clamp experiments were also carried out on thapsigargin-treated cells after they were allowed to incubate for 8 hours or 24 hours following a single thapsigargin exposure to determine how long the effect of this treatment on the expression of the CFTR-like channel could persist. After an 8 hour recovery period CFTR-like channel activity was observed in 7 of 20 excised patches (35%). However after a 24 hour recovery period 0 of 10 patches (0%) demonstrated any CFTR-like channel activity.

Treatment with calcium pump inhibitors leads to a transient rise in intracellular calcium concentrations, which has been shown to acutely stimulate chloride currents in CF epithelial cells (Chao *et al.*, J. Clin. Invest. 96:1794-1801(1995)). To ascertain if the change in CFTR channel activity was due to this short term effect of thapsigargin, cells were treated with a short exposure to thapsigargin (15 minutes) and then allowed to recover for 2 hours prior to patch clamping. No CFTR-like channel activity was stimulated in 10 patches following this protocol (data not shown), suggesting that short-term elevations of intracellular calcium concentrations that follow treatment with thapsigargin do not result in detectable long term increases in CFTR-like channel activity.

Table 1.

Cell Type	Incubation Condition	Patches with CFTR channel activity
IB3-1	control, no treatment	0/10 (0%) (in previous studies 0/35)
ΣCFBE290$^-$	control, no treatment	0/8 (0%)
IB3-1	thapsigargin treated	25/76 (32.8%)

Cell Type	Incubation Condition	Patches with CFTR channel activity
ΣCFBE290⁻	thapsigargin treated	8/24 (33.3%)
Combined	control, no treatment	0/28 (0%)
Combined	thapsigargin treated	33/100 (33%)

[1] **Note:** Normally in unaffected airway epithelial cells CFTR channel activity can be detected via patch clamp techniques in 70% of patches.

Experiment 2. Short circuit current measurements.

Materials and Methods. CFPAC-1 or T84 cells were grown on collagen coated permeable supports (Transwell Snapwell filter cups, Corning Costar, Cambridge, MA). Cells were fed every one to two days from the basolateral surface of the monolayer while the apical surface was exposed to the humidified 5% CO_2 environment. Filters were cultured until a tight monolayer was achieved.

Prior to electrical studies some of the monolayers were treated with 1uM thapsigargin using the following protocol. Culture media containing 1uM thapsigargin was added to the apical surface of the monolayer and incubated for 1.5 hours at 37°C. Cells were then rinsed with fresh thapsigargin-free media and allowed to incubate for 2 hours at 37°C, after which they were used for Ussing chamber studies. The Ussing chamber bath solution was a nominally bicarbonate-free Ringer's solution that was composed of (in mM) 140 NaCl, 1.2 $MgCl_2$, 5 K_2HPO_4, 0.5 KH_2PO_4, 5 HEPES, 1.2 $CaCl_2$, and 5 glucose pH=7.4. Bath solutions were warmed to 37°C.

Ag-AgCl wires were embedded in 3M KCl agar bridges were used as voltage and current electrodes on each side of the monolayer contained in an Ussing chamber system (World Precision Instruments, WPI). Voltage was clamped using an EC-825 voltage clamp amplifier (Warner Instruments) with a digital current and voltage readout. The transepithelial potential difference (V_{te}) is continuously recorded. At 5-minute intervals the V_{te} is clamped to 0 and the short circuit current (I_{sc}) was

determined. Under I_{sc} conditions a voltage pulse between 20 and 40 mV was applied and the change in current was used to calculate the transepithelial resistance (R_{te}).

After cells were mounted in the Ussing chamber electrical parameters were assessed for 20 to 30 minutes (control period). Following the control period a cAMP-stimulating cocktail (10μM forskolin and 100μM IBMX) was added to the apical chamber. Electrical parameters were monitored for 20-30 minutes following this treatment to assess for changes in I_{sc}, V_{te}, and R_{te}. Furosemide (10^{-4} M), an inhibitor of chloride secretion, was then added to the basolateral bath for 20 minutes to assess its affect on chloride secretion. In the continued presence of furosemide, 10^{-4}M amiloride, an inhibitor of sodium absorption, was added to the apical bath for 10 minutes. During these maneuvers, electrical parameters were continuously monitored.

Results. To determine whether the thapsigargin effect on CFTR channel activity is of sufficient magnitude to increase epithelial short circuit current, CFPAC-1 cells (Schoumacher *et al.*, Proc. Natl. Acad. Sci. 87:4012-4016 (1990)) were grown on collagen-coated permeable supports and examined in Ussing chambers. When monolayers of untreated CFPAC-1 cells were exposed to a cAMP-stimulation there was no increase in the short circuit current (-0.38±1.8 %, n=12) (Figure 3). The lack of response to the elevation of cytosolic cAMP concentrations is consistent with the CF phenotype (Grubb *et al.*, Am. J. Resp. Cell. Mol. Biol. 8:454-460 (1993)).

In contrast, when thapsigargin treated CFPAC-1 monolayers were exposed to the cAMP-stimulation cocktail, there was a 14.6± 6.6 % increase in short circuit current (n=12, p=0.02) which was inhibited by furosemide, suggesting it was due to an increase in net chloride secretion. The presence of the cAMP-stimulated chloride secretion in the thapsigargin-treated CFPAC cells is consistent with a partial correction of the CF ion transport defect and it is similar in magnitude to that seen with T84 cell monolayers (Figure 3). T84 cells are a human colonic epithelial cell line that expresses high levels of wild-type CFTR (Cohn *et al.*, Proc. Nat. Acad. Sci. 89:2340-2344.(1992); Bell and Quinton, Am. J. Physiol. 262:C555-C562.(1992)).

Experiment 3. Immunofluorescence analysis.

CFPAC and ΣCFBE290⁻ epithelial cells were grown to confluence on 0.45 μ Transwell filter inserts (Corning Costar, Cambridge, MA) under the same conditions described for the short circuit current measurements. Prior to immunofluorescence analysis, filter grown cell monolayers were treated for 90 min with 1 μM thapsigargin at 37°C, present in both the apical and basolateral media compartments. The media was